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An Ultrastructural Investigation of Spermatogenesis in the Holopelagic Polychaetes *Vanadis formosa* and *Krohnia lepidota* (Polychaeta: Alciopidae)

STANLEY A. RICE[1] AND KEVIN J. ECKELBARGER[2]

[1]*Division of Science and Mathematics, University of Tampa, Tampa, Florida 33606, and*
[2]*Reproductive Biology Program, Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Fort Pierce, Florida 34946*

Abstract. The earliest stages encountered were clusters of spermatogonial cells floating free in the coelom. Neither germinal epithelium nor definitive gonad was observed. Mitotic divisions of spermatogonial cells resulted in large coelomic clusters containing up to 800 cells each. Cells within these clusters were connected to neighboring cells via cytoplasmic bridges that were characterized by a dense collar around the cell membrane and by microtubules traversing the common cytoplasm between cells. Peripheral cells detached from large cell clusters and entered meiotic divisions in the coelom. Spermatid nuclei initially contained granular chromatin that became fibrillar and eventually electron opaque as nuclear elongation and condensation proceeded. The acrosome first appeared in the posterior cytoplasm as a proacrosomal vesicle with an associated Golgi complex. The plate-like proacrosome migrated toward the anterior end of the sperm and reached the apex prior to completion of nuclear condensation. The middlepiece contained 5–7 mitochondria in *Vanadis formosa* and 8–10 mitochondria in *Krohnia lepidota*. Two centrioles, perpendicular to each other, were located in the middlepiece with the proximal centriole residing in a shallow fossa at the base of the nucleus. The distal centriole was associated with a nine-spoke, branched anchoring apparatus at the posterior edge of the middlepiece. Sperm development and mature sperm ultrastructure were very similar in *V. formosa* and *K. lepidota*. The mature coelomic sperm of *K. lepidota* were longer than those of *V. formosa* (8.7 μm *vs.* 11.0 μm, nucleus + middlepiece). The posterior nuclear region of *V. formosa* sperm was irregular in shape while that of *K. lepidota* was symmetrical. A series of dense accessory "membranes" was observed between the nucleus and the plasmalemma of *K. lepidota* sperm but not in *V. formosa*. Stored sperm in female worms were observed in *V. formosa* but no female *K. lepidota* were available in this study. Sperm storage and elongated sperm structure in alciopid polychaetes may be related to the pelagic environment in which they live and the suspected infrequent encounters between individuals.

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Introduction

The Alciopidae is one of six holopelagic polychaete families whose representatives spend their entire life history in the water column (Dales and Peter, 1972). Alciopids have evolved a number of specialized features for pelagic existence including two large, highly developed eyes, long thin transparent bodies, and specialized reproductive characteristics. The structure and function of the complex eyes of alciopids have been described by Hermans and Eakin (1974) and Wald and Rayport (1977). The fragility of alciopids and the problems associated with collection of healthy, intact individuals have precluded detailed ultrastructural studies of gametogenesis until recently. Rice (1980, 1984, 1987) has reported on the *in situ* behavior, reproductive biology, and evolution of 14 alciopid species from the western Atlantic Ocean. Eckelbarger and Rice (1988) have described the ultrastructural events of oogenesis in two species of alciopids.

Alciopid polychaetes are believed to have arisen from ancestral stock similar to the present-day phyllodocid,


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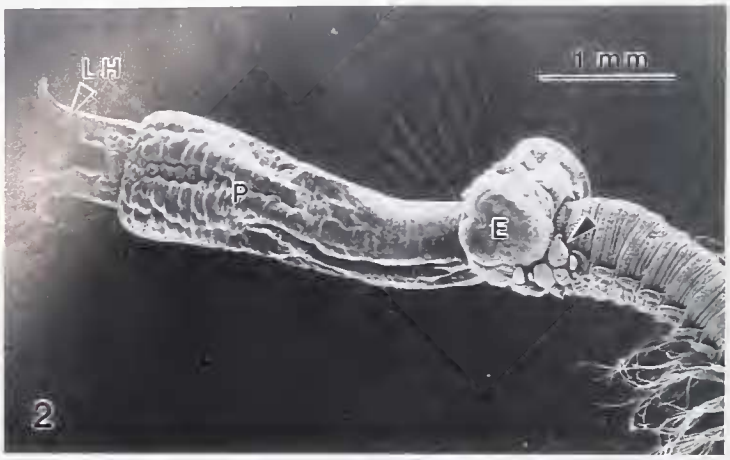
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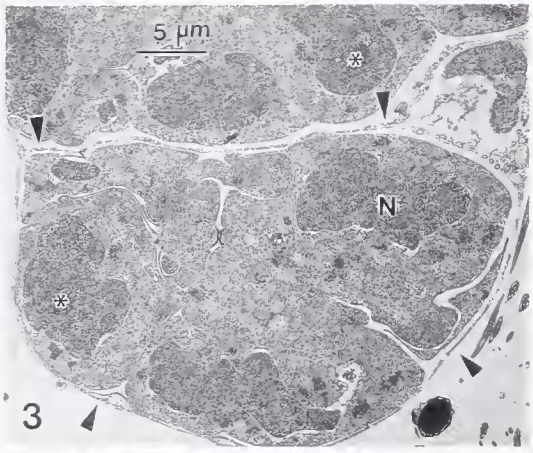
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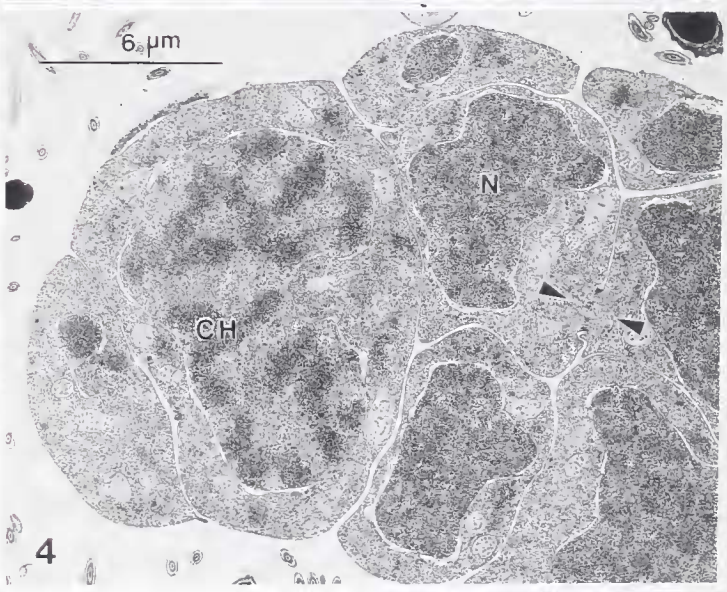
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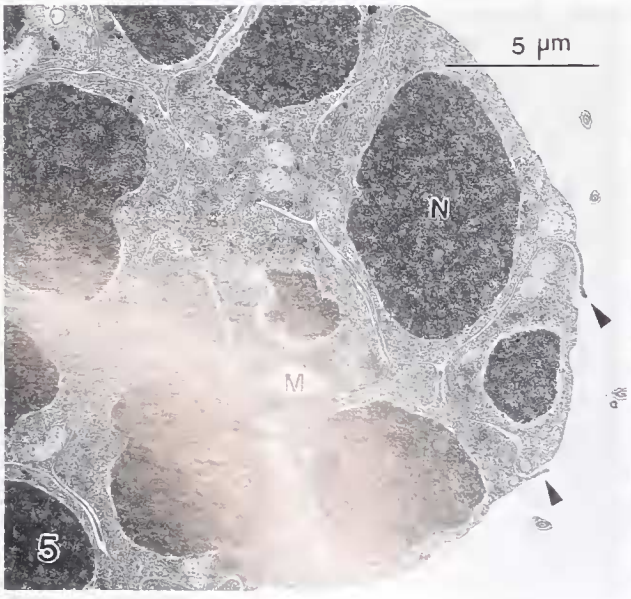
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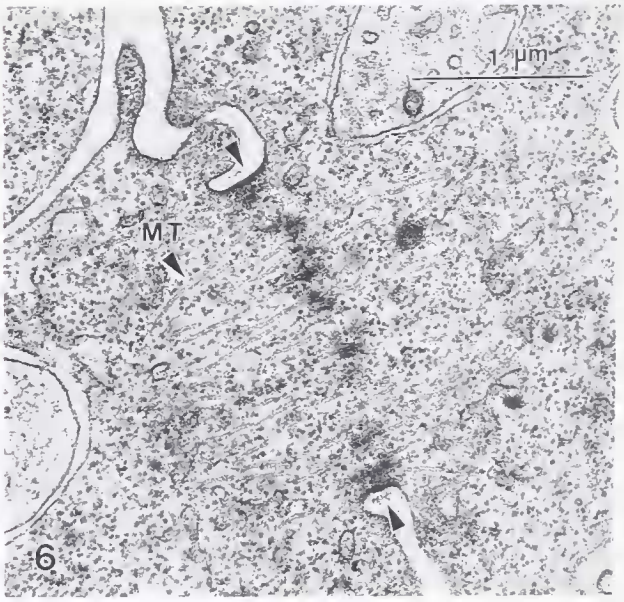
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Eulalia (Dales, 1955; Ushakov, 1972). Detailed ultrastructural information on alciopids will be central to testing this hypothesis of evolutionary descent of the family and also for establishing generic relationships within the Alciopidae.

Spermatogenesis has not been previously described in any species of Alciopidae. Rice (1987) presented information on the external morphology of mature sperm from eight genera and indicated that considerable diversity exists between sperm from different genera within the family. Franzén and Rice (1988) presented micrographs of the mature sperm of *Naiades cantrainii, Torrea candida,* and *Krohnia lepidota* in their review of polychaete sperm ultrastructure but did not describe these sperm or their development in detail. Studies of sperm morphology and sperm development have proven useful in other polychaetes in analyzing systematic relationships and phylogenetic position (Rice, 1981; Pfannensteil *et al.,* 1987; Eckelbarger and Grassle, 1987). The Alciopidae has been poorly studied compared to other polychaete families and the present study was intended to provide new information that may have implications for the generic relations within the family as well as the relationship between the Alciopidae and other polychaete families. The variety of reproductive adaptations seen in the Alciopidae suggests a possible evolutionary scheme that may be shared by other pelagic animals.

Materials and Methods

The animals in this study were collected (by S.A.R.) from the Northwest Providence Channel, Bahama Islands, using a JOHNSON-SEA-LINK submersible operated by Harbor Branch Oceanographic Institution. Collections were made in September and November, 1979, and in April, 1980, with both *Krohnia lepidota* (Krohn, 1845) and *Vanadis formosa* Claparede, 1870, collected on each occasion. *Vanadis formosa* (Fig. 2) was the most abundant species encountered of the 14 species collected (Rice, 1987), with a total of 59 specimens collected at depths of 80–180 m. *Krohnia lepidota* (Fig. 1) was encountered only occasionally with a total of 12 specimens collected at depths of 90–150 m.

The majority of *Vanadis formosa* collected were males with an overall sex ratio of 6:1. All specimens of *Krohnia lepidota* collected were males, although this probably reflects unavoidable sampling error due to the limitations of the collecting techniques and the small number of individuals collected.

Living animals were captured and returned to the surface using the specialized zooplankton collection device on the JOHNSON-SEA-LINK. Specimens were fixed within one hour of collection for scanning (SEM) and transmission (TEM) electron microscopy. Specimens for TEM were fixed for two hours at room temperature (23–25°C) in 2.5% glutaraldehyde buffered with 0.2 *M* sodium phosphate followed by postfixation for 1 h in 1% osmium tetroxide buffered with 1.25% sodium bicarbonate. Specimens were identified following postfixation, then dehydrated in increasing concentrations of ethanol, transferred through two changes of propylene oxide, and embedded in Epon. Sections were cut on a Porter-Blum MT-2B ultramicrotome with a diamond knife, stained with aqueous saturated uranyl acetate and lead citrate, and examined with a Zeiss EM9-S2 transmission electron microscope. SEM specimens were fixed as above followed by dehydration to amyl acetate. Critical point drying was accomplished using liquid CO_2 as the transition solvent. Dried specimens were mounted and coated with gold-palladium and viewed with a Zeiss Novascan 30 scanning electron microscope.

Results

Spermatogenesis

Spermatogenesis in *V. formosa* and *K. lepidota* occurs in the spacious coelomic cavity of the animal. No gonad was found in either species and no conclusive ultrastructural evidence confirming the sequence of mitotic and meiotic divisions was encountered. It is proposed that clusters of spermatogonial cells arise from the coelomic

Figure 1. Lateral view of head of *Krohnia lepidota* viewed with SEM. E, eye; MA, median antenna.

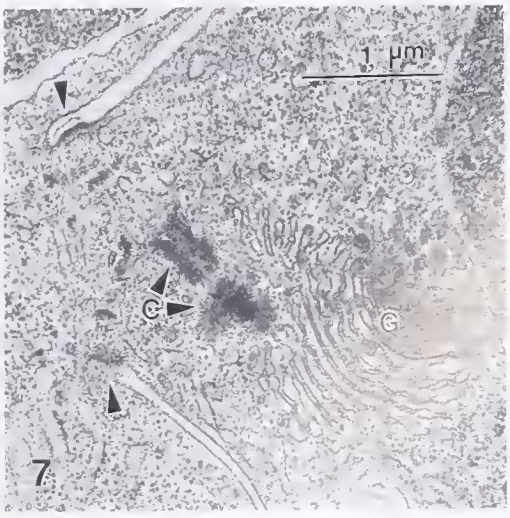
Figure 2. Lateral view of head of adult female *Vanadis formosa* viewed with SEM. E, eye; P, extended proboscis; LH, lateral horn of proboscis. Arrow indicates seminal receptacle.

Figure 3. Clusters of spermatogonia with irregular nuclei (N) and small nucleoli (*) in *V. formosa*. Note thin layer of sheath cells surrounding cell clump (arrows).

Figure 4. Cluster of spermatocytes showing irregular nuclei (N) and intercellular bridge (between arrows) in *V. formosa*. Note chromosome figures (CH) in cell at left.

Figure 5. Cluster of spermatocytes with densely staining nuclei (N), small cell extensions (arrows), and mitochondria (M) in *V. formosa*.

Figure 6. High magnification of intercellular bridge between adjacent *V. formosa* spermatocytes. Note numerous microtubules (MT) and electron dense thickening of bridge plasmalemma (arrows).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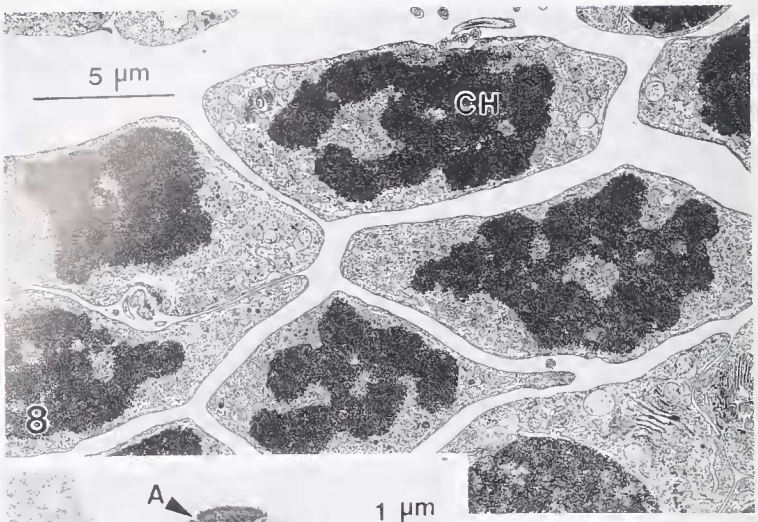
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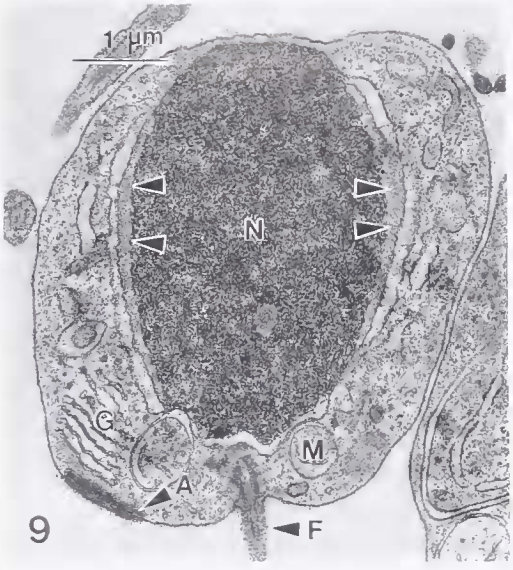
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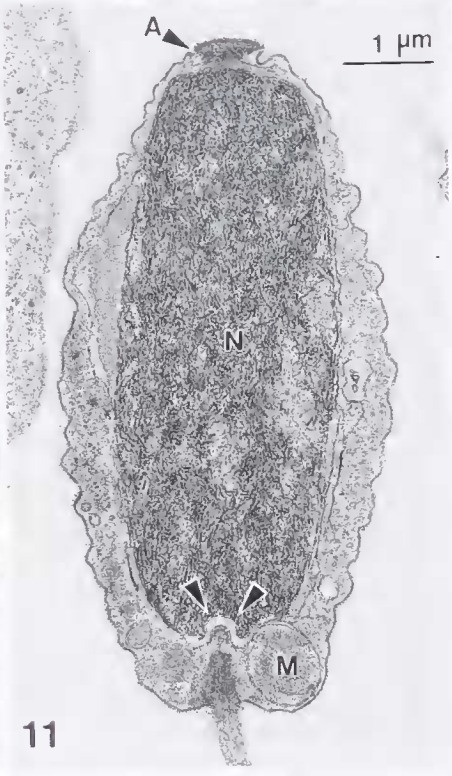
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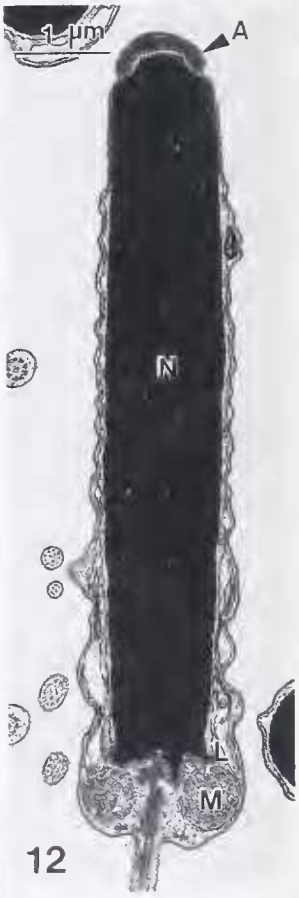
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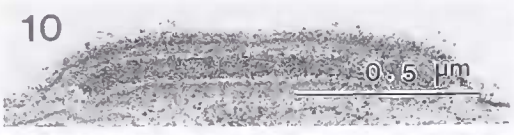
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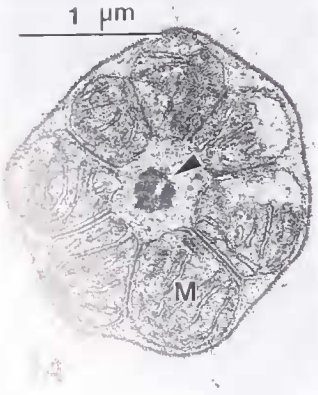
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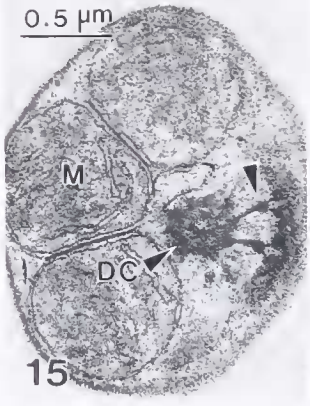
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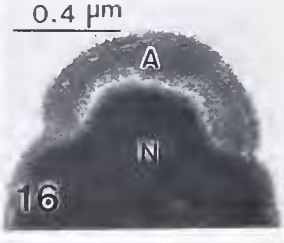
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epithelium and undergo numerous mitotic divisions in the coelom followed by meiotic divisions and terminal differentiation of peripheral cells in the larger cell clusters. Spermatids detach from these cell clusters and mature singly in the coelom in *V. formosa* or in groups in *K. lepidota*. The designation of spermatogenic stages in the following account is based upon the size of cell clusters, size and morphology of nuclei, and the appearance of characteristic structures such as centrioles and flagellum.

The following account of spermatogenesis is based primarily upon specimens of *V. formosa* for which the most material was available. The sequence of events and the ultrastructure of sperm development are very similar in *V. formosa* and *K. lepidota*. It may be assumed that the following descriptions apply to both species unless otherwise specified. Cellular measurements refer to *V. formosa* unless specifically attributed to *K. lepidota*.

Proliferating spermatogonia

The earliest stages encountered in the coelom are small spermatogonial clusters of 8–10 cells that are surrounded by sheath cells presumably derived from the coelomic peritoneum (Fig. 3). In some instances, several small clusters of proliferating cells are surrounded by a common cellular envelope of sheath cells. The nuclei of these spermatogonial cells are irregular in shape with an average diameter of 7.2 μm (n = 37). The chromatin is granular and heterogeneous in non-dividing nuclei (Fig. 4). Chromosome figures are present within the nuclei of some cells (Fig. 4). In later proliferating stages, the nuclei become more electron-dense and more spherical in shape (Fig. 5). Cytoplasmic volume is about equal to, or less than, nuclear volume during interphase with prominent intercellular bridges joining up to four adjacent cells. Intercellular bridges are characterized by a collar of electron-dense material and by the presence of numerous microtubules crossing the bridges along the axial plane (Fig. 6). Two centrioles, oriented perpendicular to each other are present near intercellular bridges (Fig. 7). Numerous mitochondria are present throughout the cytoplasm along with well-developed Golgi cisternae (Figs. 5, 7).

Repeated mitotic divisions produce coelomic cell clusters up to 0.8 by 0.4 mm that contain approximately 800 cells. Nuclear diameter averages 5.9 μm (n = 30) in these cells. The sheath cells are absent after the early stages of proliferation although cytoplasmic extensions of peripheral cells in larger clusters gives the appearance of a sheath cell covering (Fig. 5).

Spermatocytes and early spermatids

Chromosome figures are commonly observed in cells at the periphery of larger spermatogonial cell clusters (Fig. 8). These peripheral cells become detached from the cluster and lose cytoplasmic continuity with adjacent cells. It is likely that these dividing cells represent primary and secondary spermatocytes undergoing meiosis.

In *Vanadis formosa*, cytoplasmic bridges are infrequently encountered between cells that have detached from the large cell clusters. However, in *Krohnia lepidota*, groups of four spermatids are connected by cytoplasmic bridges. These groups of four cells are likely the result of the two meiotic divisions of spermatogonial cells.

Following the presumptive meiotic divisions, the nuclei become spherical again with an average diameter of 3.9 μm (n = 3). The chromatin of these early spermatids

Figure 7. Intercellular bridge (between arrows) in *Vanadis formosa* with adjacent centrioles (C) and Golgi complex (G) in developing spermatocytes.

Figure 8. Chromosome figures in dividing *V. formosa* spermatocytes.

Figure 9. Early *V. formosa* spermatid with oval nucleus (N) containing granular chromatin and lateral regions of uncondensed nucleoplasm (arrows). Note Golgi complex (G) and adjacent proacrosome (A), mitochondrion (M), and flagellum (F).

Figure 10. Higher magnification of proacrosome from Figure 9.

Figure 11. Elongating spermatid of *V. formosa* showing filamentous chromatin in nucleus (N), developing nuclear fossa at base of nucleus (arrows), and terminal acrosome (A).

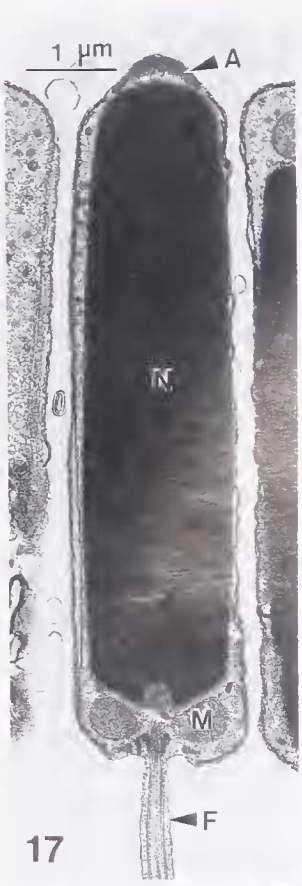
Figure 12. Mature spermatozoan of *V. formosa*. A, acrosome; N, nucleus; M, mitochondrion.

Figure 13. Higher magnification of middlepiece region of mature spermatozoan. Note the asymmetry of the posterior region of the nucleus (N). DC, distal centriole; PC, proximal centriole; F, flagellum; M, mitochondrion.

Figure 14. Cross section through middlepiece of mature *V. formosa* spermatozoan showing ring of mitochondria (M) and centrally positioned distal centriole (arrow).

Figure 15. Tangential section through middlepiece of mature *V. formosa* spermatozoan showing mitochondria (M) and distal centriole with a portion of the centriolar anchoring devise (arrows).

Figure 16. High magnification of acrosome (A) and anterior portion of the nucleus (N) in *V. formosa* mature spermatozoan.



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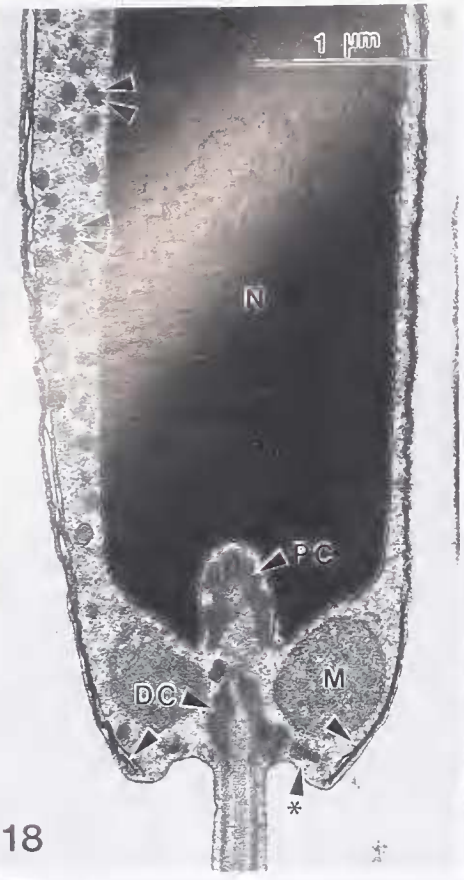

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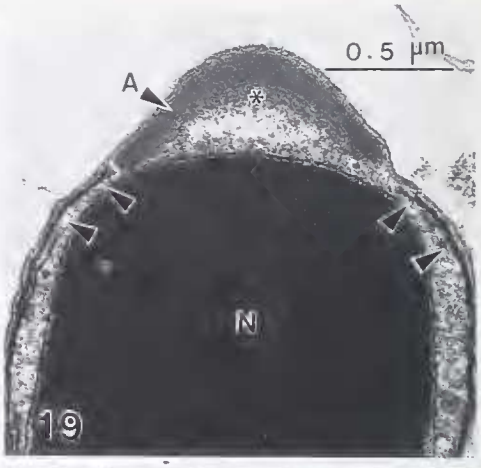

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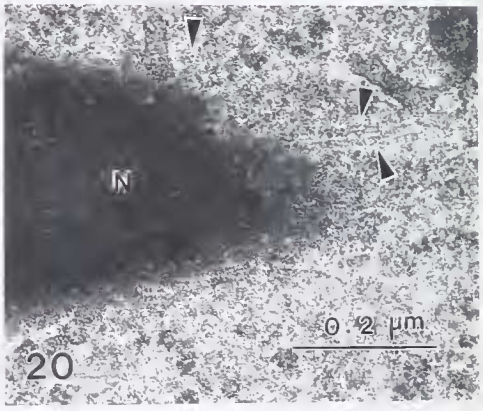

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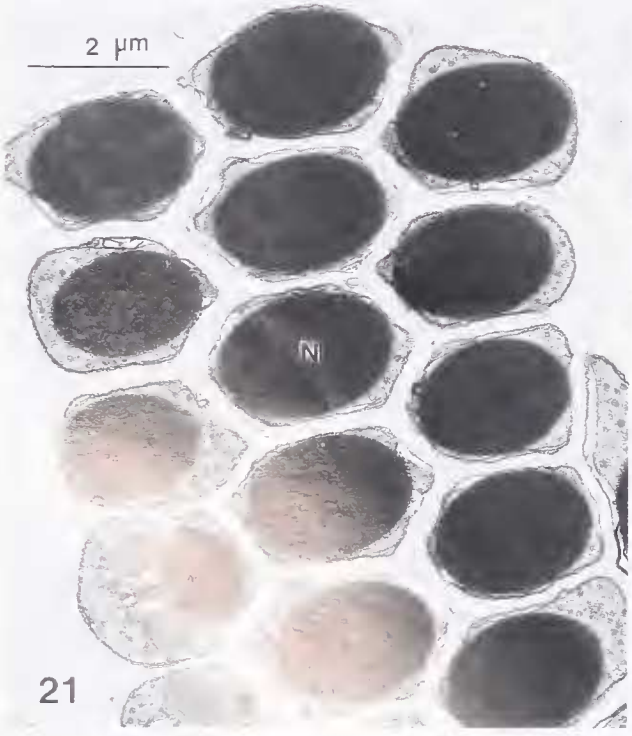

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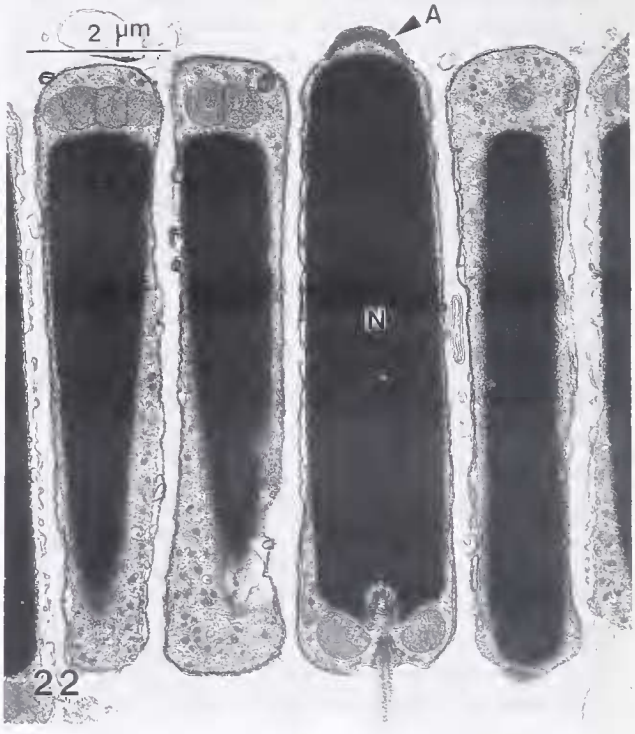

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appears coarsely granular with mottled patches of electron-transluscent material especially along the peripheral region of the nucleus (Fig. 9). Two centrioles are present and the sperm flagellum begins to form signaling the beginning of terminal differentiation of the cell. Four to seven large mitochondria accumulate at the posterior end of the spermatids prior to nuclear elongation and surround the developing centriolar complex of the flagellum (Fig. 9). The proacrosome and a single Golgi complex are located in the posteriolateral portion of the cell near the flagellum (Fig. 9). The proacrosome is membrane-bounded and closely applied to the plasma membrane of the cell. Its contents are homogeneous and electron-dense (Fig. 10).

Late spermatids

The most conspicuous events that distinguish early from late spermatids are the location of the proacrosomal vesicle, elongation of the nucleus, formation of the centriolar fossa at the base of the nucleus, and a textural change in the chromatin from granular to fibrillar in late spermatids.

The proacrosomal vesicle is positioned at the anterior end of the sperm while maintaining close contact with the plasmalemma. The vesicle is plate-like in shape with a flattened appearance in cross section (Figs. 10, 11). The Golgi complex that was initially associated with the acrosomal vesicle is no longer visible.

The nucleus elongates progressively reaching a length of 5.6 μm when the proacrosome reaches the apex of the spermatid. In favorable sections, a manchette of microtubules surrounds the spermatid nucleus. At the posterior end of the nucleus, a symmetrical fossa containing the proximal centriole forms (Fig. 11).

Chromatin condensation is characterized by the appearance of electron-dense fibers interspersed with patches of less-dense nucleoplasm. In cross section, the chromatin fibers measure 30 nm and are generally oriented parallel to the long axis of the nucleus (Fig. 11). An inflated nuclear envelope (or perinuclear cisternae, from the older literature) is characteristic of this stage. The chromatin fibers become progressively more densely packed and thus electron-opaque as the nucleus completes elongation.

Mature coelomic sperm of Vanadis formosa

The mature coelomic sperm (Fig. 12) has a homogeneously electron-dense nucleus with an average length of 7.7 μm (n = 6). The nucleus is slightly fusiform in shape with a diameter of 1.4 μm (n = 6) near the midpoint. The acrosome forms a cap, about 1 μm in diameter, over the tip of the nucleus (Fig. 16). A constriction at the anterior tip of the nucleus forms a ledge upon which the acrosome rests giving the anterior end of the sperm a smooth to slightly indented outline (Fig. 16). Nuclear material extends into the convex space below the acrosome. The acrosomal contents are homogeneous and less electron-dense than the nucleus. The acrosome is bounded by an acrosomal membrane and overlain by the closely apposed sperm plasma membrane. The plasmalemma has an irregular outline over all but the most anterior region of the cell, which may represent a fixation artifact (Fig. 12). The posterior portion of the nucleus is irregularly shaped with a displaced centriolar fossa measuring 1.2 by 1.0 μm and containing the proximal centriole (Fig. 13).

The middlepiece is short compared to the nucleus with an average length of 1.0 μm (n = 5) and contains 5–7 spherical mitochondria surrounding the centriolar complex at the base of the nucleus (Fig. 14). Mitochondrial diameter averages 0.7 μm (n = 2) with a maximum diameter of 0.9 μm. The centriolar complex is composed of a proximal centriole located at the base of the nucleus and a distal centriole located between the mitochondria and continuous with the sperm flagellum (Fig. 13). The proximal centriole contains nine triplets of microtubules and

Figure 17. Mature spermatozoan of *Krohnia lepidota*. A, acrosome; N, nucleus; M, mitochondrion; F, flagellum.

Figure 18. Posterior half of mature *K. lepidota* spermatozoan showing proximal centriole inserted into symmetrical fossa at base of nucleus (N). Note accessory membranes beneath lateral plasmalemma (single arrows) and electron dense material (*) associated with the distal centriole (DC) posterior to the mitochondria (M). The sperm cytoplasm contains numerous membrane-bounded inclusions (double arrows).

Figure 19. Anterior region of mature *K. lepidota* spermatozoan showing acrosome (A), subacrosomal material (*), and accessory membranes (double arrows) beneath the plasmalemma. N, nucleus.

Figure 20. Tangential section through nuclear region of *K. lepidota* spermatozoan showing microtubular manchette (arrows) running parallel to the long axis of the nucleus (N).

Figure 21. Cross section through coelomic sperm packet in *K. lepidota*. Note regular spacing between adjacent sperm.

Figure 22. Longitudinal section through coelomic sperm packet in *K. lepidota*. Note the alternating orientation of sperm in the packet.

is positioned perpendicular to the long axis of the sperm in a shallow centriolar fossa. The distal centriole also contains nine triplets of microtubules as well as nine lateral, branching extensions forming an anchoring structure at the base of the flagellum (Fig. 15).

Mature coelomic sperm of Krohnia lepidota

Although the general shape of the coelomic sperm is similar between the two species, the mature coelomic sperm of *Krohnia lepidota* differ from those of *Vanadis formosa* in size, organelle shape, cytoplasmic inclusions, and the presence of accessory membranes. The head of the mature coelomic sperm of *K. lepidota* (Fig. 17) measure 11.0 μm (n = 5) in length (acrosome + nucleus + middlepiece). The acrosome is cap-like with a diameter of 1.2 μm (n = 10) and a subacrosomal space filled with a flocculent material (Fig. 19). In favorable sections, a manchette of microtubules is observed parallel to the nucleus (Fig. 20). The nucleus measures 7.7 μm in length (n = 7) with a maximum diameter of 1.7 μm (n = 22). A thin layer of cytoplasm surrounds the nucleus and middlepiece and contains numerous spherical, membrane-bounded inclusions of variable electron density and unknown composition (Fig. 18). These inclusions range in size from 0.08–0.14 μm (mean = 0.11, n = 14).

The middlepiece is short (ca. 2 μm) as in *Vanadis formosa*, and contains 8–10 spherical mitochondria that average 0.7 μm in diameter (n = 10). A centriolar fossa extends about 0.5 μm into the posterior portion of the nucleus and contains the proximal centriole (Fig. 18). The distal centriole is located between the mitochondria and is attached to a nine-spoke flagellar anchoring structure similar to that of *Vanadis formosa* described above (Fig. 18).

The nucleus and middlepiece are partially surrounded by a series of electron-dense accessory "membranes" lying just beneath the sperm plasmalemma (Figs. 18, 19). The "membranes" appear to be composed of a layer of electron-dense material and are absent from the extreme anterior and posterior regions of the sperm head (Figs. 18, 19). They are thicker and stain more densely than the nuclear envelope or the plasmalemma. As many as three "membranes" are present in some sperm between the plasmalemma and the nuclear envelope.

The coelomic sperm of *K. lepidota* often occur in packets of 18 or more cells oriented parallel to each other (Fig. 21, 22). No cytoplasmic connections are visible between cells to account for the apparent cohesiveness of these packets. In longitudinal sections, the sperm alternate with regard to anterior-posterior orientation (Fig. 22).

Hermaphroditic Vanadis formosa

Two out of 59 specimens of *Vanadis formosa* examined contained both sperm and eggs within the coelomic compartment. Numerous stages of sperm development from early spermatogonia to late spermatids were present while egg development appeared to be in early stages only. Egg diameters ranged from 31 to 78 μm, corresponding to the previtellogenic and early vitellogenic stages of other alciopid polychaetes. Both sperm and eggs appear to be normal with no signs of gamete resorption that might indicate a progressive sex reversal.

Sperm storage by females

Vanadis formosa females store sperm within modified dorsal cirri of the first and second post-head segments (Figs. 2, 23, 24). A small pore on the ventral surface of the receptacle serves as the entry and exit site for the sperm (Fig. 24). The wall of the receptacle appears to be composed of two cell layers (Fig. 24) with the sperm heads embedded in the inner cell layer (Fig. 25). The sperm within these seminal receptacles are morphologically similar to what we consider to be mature sperm within the coelom of the males. The acrosomes of stored sperm are unreacted while the nuclear region is surrounded by numerous microvilli extending from maternal cells (Fig. 26). No intercellular junctions are visible between maternal tissues and sperm. The number of sperm contained within seminal receptacles varies between individual females and with the size of the storage organ. The ultrastructural details of sperm storage in alciopid polychaetes will be reported elsewhere.

Discussion

Vanadis formosa and *Krohnia lepidota* are among the most commonly encountered cosmopolitan alciopids in tropical and temperate seas (Tebble, 1960, 1962; Day, 1967; Dales and Peter, 1971). However, intact specimens suitable for TEM studies are difficult to obtain due to the fragile structure of most alciopids. These animals have not been successfully maintained in the laboratory and thus unanswered questions remain concerning their biology. For example, many alciopids, including *V. formosa* and *K. lepidota*, are transparent when alive but have never been observed to be full of gametes. The coelomic cavity is spacious with relatively few gametes present, relative to available space, as compared to other polychaetes of comparable size. This may be due to a sampling artifact in the number of individuals collected or may represent an adaptive strategy for increased transparence (see below). The length of the gametogenic cycle and the possibility of consecutive hermaphroditism are

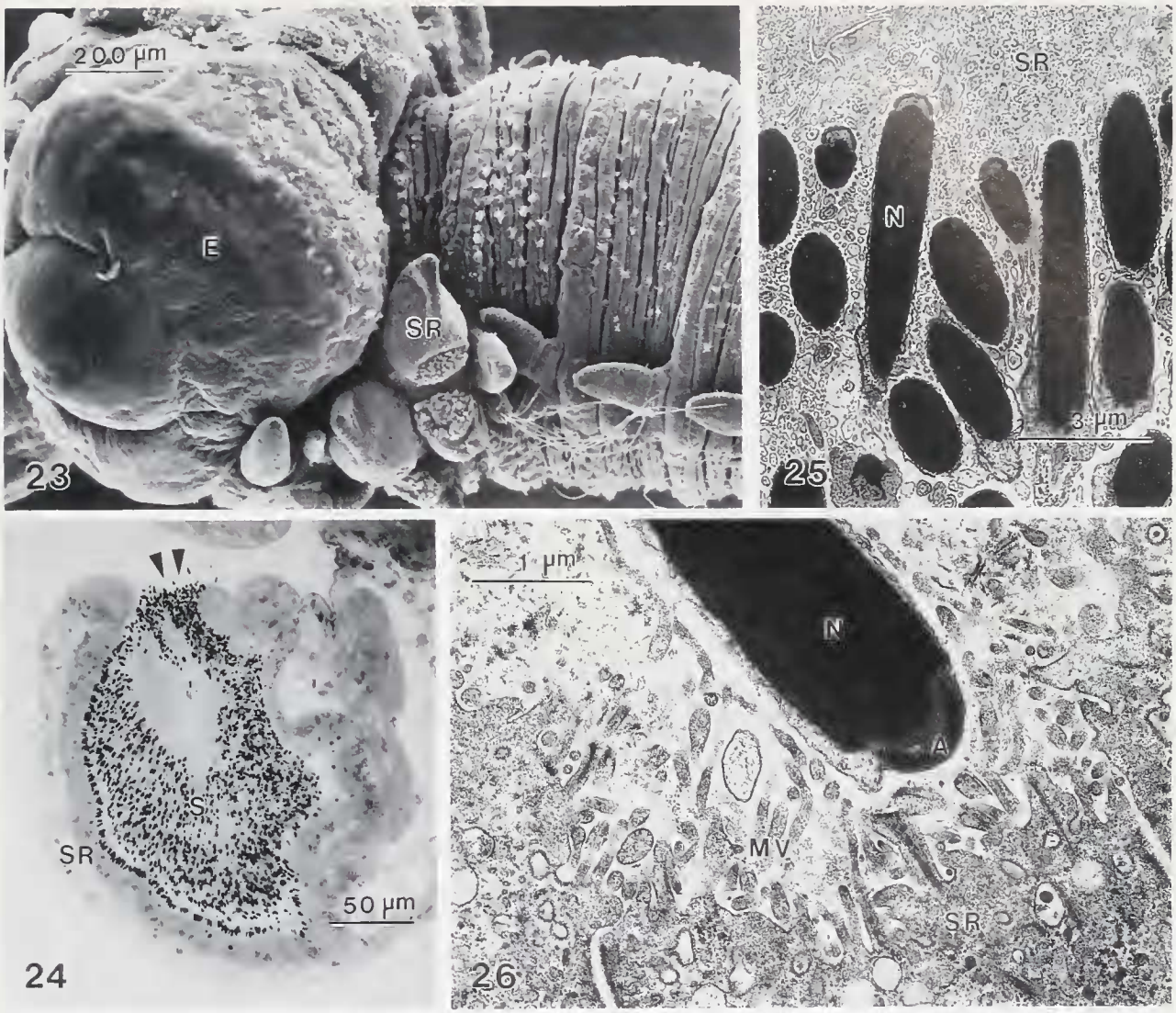

Figure 23. Scanning electron micrograph showing lateral view of head in female *Vanadis formosa*. Note the enlarged dorsal cirri of the first two podia, just behind the eye (E), that serve as seminal receptacles (SR).

Figure 24. Light micrograph of a one micron thick section through one of the seminal receptacles (SR) showing enclosed sperm (S) and pore leading to the exterior (double arrows).

Figure 25. Longitudinal section of mature *V. formosa* sperm embedded in the wall of the seminal receptacle. Note that the sperm nuclei (N) are oriented at various angles within the cells of the receptacle wall (SR).

Figure 26. Higher magnification of sperm nucleus (N) and acrosome (A) surrounded by microvilli (MV) of the seminal receptacle cells (SR). Note that the sperm acrosome is unreacted and that there appear to be no cell junctions between sperm and maternal cells.

also difficult to analyze without laboratory data on living specimens.

The sperm of alciopids vary in general morphology from short, fusiform "primitive" ones (Franzén, 1956) to elongated types more characteristic of "modified" sperm (Rice, 1987). Both *V. formosa* and *K. lepidota* have sperm with elongated nuclei and short middlepieces. In size and general morphology, the sperm of these two species appear very similar suggesting that (1) *Vanadis* and *Krohnia* are closely related genera or (2) convergent evolution of sperm structure has occurred. Rice (1987) applied a Pearson product-moment correlation coefficient analysis to the nine alciopid genera and found that *Vanadis* and *Krohnia* were not closely related (similarity

less than 25%) based upon ten morphological and reproductive characteristics. It is likely that the similarity of the sperm in these two species is more closely related to reproductive biology than to recent common ancestry.

The primordial germ cells and gonads of most polychaetes are associated with the coelomic epithelium or the intestinal/nephridial blood vessel epithelium (Olive, 1983; Eckelbarger, 1984; Sawada, 1984). In *Vanadis formosa* and *Krohnia lepidota*, no gonads or primordial germ cells were observed in these traditional locations. In the alciopids *Rhynchonerella angelini* and *Alciopa reynaudii*, Eckelbarger and Rice (1988) found no definitive ovary and reported that the earliest stages observed were coelomic oogonia.

There is a high degree of similarity in sperm development between the families Nereidae, Phyllodocidae, and Alciopidae, which is consistent with their traditional systematic grouping (Fauchald, 1977). In the phyllodocid *Eulalia viridis*, Olive (1975) reported that a "dispersed gonad" exists in which free-floating coelomic cells of uncertain origin give rise to the gametes. These coelomic germ cells then produce large packets of spermatogonia, containing thousands of cells, and eventually disperse as rosettes of primary and secondary spermatocytes. In the Nereidae, the location of definitive gonads has also proven illusive (Eckelbarger, 1984; Sawada, 1984). The earliest spermatogenic stage reported for *Perinereis brevicirris* was coelomic cell clusters of several hundred spermatogonia that appeared to be subdivided by cytoplasmic processes into blocks of several dozen cells each (Kubo and Sawada, 1977). In *Platynereis dumerilii* and *P. massiliensis*, proliferating spermatogonia appear from an unidentified germinal epithelium as free-floating clusters of 60 or more cells that are enveloped by sheath cells (Pfannenstiel *et al.*, 1987). The development of spermatogonial clusters within the coelomic cavity is not unique to these three families however, with similar processes reported for *Pomatodrilus fluviatilis* (Bunke, 1985) and for *Nicolea zostericola* (Eckelbarger, 1975) although in both of these species, the germ cells could be traced back to a distinct gonad.

The sequence of the meiotic and mitotic divisions during sperm development could not be established with certainty in the present study. However, in other polychaetes the presence of synaptonemal complexes or other indicators of meiosis have been reported to occur as peripheral cells detach from coelomic spermatogonial clusters (Olive, 1975; Kubo and Sawada, 1977; Sawada, 1984). It is likely that this occurs in *V. formosa* and *K. lepidota*.

Spermiogenesis in *V. formosa* and *K. lepidota* is similar to that described for other polychaetes. The condensation of chromatin follows the fibrillar model and the nucleus is surrounded by a manchette of microtubules (see Sawada, 1984, and Franzén and Rice, 1988, for reviews). It has been suggested that the microtubules associated with the nucleus during condensation and elongation in *Spirorbis morchi* are directly involved in shaping the nucleus (Potswald, 1967). In other polychaete species however, nuclear condensation and elongation proceed without the assistance of microtubules (Rice, 1981).

The acrosomes of *V. formosa* and *K. lepidota* are typical of the Alciopidae and relatively simple in structure. With the exception of *Torrea candida*, which has a pointed conical acrosome (Rice, 1987), and *Alciopina parasitica*, which has a complex elongated acrosome (Rice, unpub. obs.), all alciopid species examined have a simple cap-like acrosome similar to *V. formosa* and *K. lepidota* (Rice, 1984, 1987). In *Eulalia* sp., the only phyllodocid for which ultrastructural information is available, the acrosome is simple and cap-like (Rouse, 1986, 1988).

The irregular shape of the posterior nucleus in mature *Vanadis formosa* sperm is relatively unusual in polychaetes. Typically, the posterior portion of the nucleus is rounded with an implantation fossa containing the centriolar complex (Sawada, 1984; Franzén and Rice, 1988). It is not uncommon for the posterior nucleus of polychaete sperm to have symmetrical indentations where it rests against the mitochondria as in *Naiades cantrainii* and *Branchiomma bombyx* (Franzén and Rice, 1988). The irregular shape of the posterior nucleus in *V. formosa* imparts an asymmetry to the sperm with respect to the centriolar complex and flagellum. This asymmetry would likely cause the sperm to rotate while swimming. A similar asymmetry has been reported in the sperm of *Exogone gemmifera* (Franzén, 1956), *Chitinopoma serrula* and *Capitella capitata* (Franzén, 1982; Eckelbarger and Grassle, 1987), and *Phragmatopoma lapidosa* (Eckelbarger, 1984). The offset flagellum in *P. lapidosa* may counter the effects of the elongated, curved acrosome to stabilize the sperm during swimming (Eckelbarger, 1984).

The accessory "membranes" observed in the sperm of *Krohnia lepidota* are unique within the Polychaeta. Franzén (1982) observed electron-dense layers between the nucleus and plasmalemma of *Autolytus* sp. sperm but determined these to be flagellar rootlets eminating from the centriolar complex. He suggested that these rootlets functioned in anchoring the flagellum to the sperm cell. The accessory membranes in *K. lepidota* sperm may represent an artifact of fixation, although we have seen no comparable structures in dozens of other polychaete species fixed under similar conditions.

Olive (1983) and Franzén and Rice (1988) have reviewed the hypothesis that elongated or modified sperm

in the Polychaeta are associated with specialized mechanisms of sperm transfer, sperm storage, or fertilization biology. In the Alciopidae, modified as well as primitive sperm types are found (Rice, 1987). Rice (1984) classified the sperm of both *V. formosa* and *K. lepidota* as modified based upon their elongated nuclei. Sperm storage in specialized seminal receptacles is known for *V. formosa* and the elongation of the sperm may represent an adaptation for sperm storage as in spionid polychaetes (Rice, 1981). However, in *K. lepidota*, Stöp-Bowitz (1948) reported that sperm may be carried between pedal lobe and ventral cirrus on the exterior of the female. No mature females were collected in the present study and thus sperm storage in *K. lepidota* could not be confirmed. Eckelbarger and Rice (1988) and Rice (1987) have confirmed external storage of sperm in the alciopids *Rhynchonerella angelini* and *R. moebii*, although both species have short, primitive sperm. Two other genera of alciopids, *Torrea* and *Naiades*, have specialized internal sperm storage organs in the females while sperm morphology is basically of the primitive type.

The ability of female worms to store sperm has been viewed as a possible adaptation to pelagic life in the Alciopidae (Rice, 1987). Six of the nine known genera in this family display some form of sperm storage in the female. With relatively low population densities (Rice, pers. obs.) and vast amounts of available habitat, it would seem advantageous for these worms to be able to take advantage of infrequent sexual encounters through transfer and storage of sperm. An evolutionary sequence of sperm storage can be envisioned leading from broadcast spawning without sperm storage, (phyllodocids), to external sperm storage of "primitive type" sperm on females, (*Rhynchonerella*), to internal storage of "primitive type" sperm, (*Torrea* and *Naiades*), and finally to internal storage by females of modified sperm, (*Vanadis*). This sequence suggests that other pelagic animals that arose from a benthic, broadcast-spawning stock might tend toward modified sperm structure and sperm storage as increasing adaptations to pelagic life.

An alternative method of minimizing the problem of infrequent sexual encounters in the open ocean would be to develop hermaphroditism (Harbison and Miller, 1986). Out of 110 alciopids representing 13 species, we have encountered only two specimens, both *V. formosa*, that were simultaneous hermaphrodites. The possibility of consecutive hermaphroditism in the Alciopidae exists, but perhaps the development of sperm storage mechanisms has been the major evolutionary thrust within the family to overcome their suspected infrequent encounters with potential reproductive partners.

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